

Zinc Supplementation of Pregnant Rats with Adequate Zinc Nutriture Suppresses Immune Functions in Their Offspring¹

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Abstract

The knowledge about consequences of marginal zinc (Zn) deficiency and Zn supplementation during pregnancy on immune function in the offspring is limited. The aim of this study was to examine whether effects of mild Zn deficiency and subsequent Zn supplementation during pregnancy persist after weaning and affect immune function of the offspring. Adult female rats were fed a Zn-adequate diet (ZC, $n = 8$) or a Zn-deficient diet (ZD, $n = 8$) from preconception through lactation. Pregnant rats were supplemented with either Zn (1.5 mg Zn in water) or placebo (water) 3 times/wk throughout pregnancy. Pups were orally immunized with cholera toxin and bovine serum albumin-dinitrophenol (DNP) 3 times at weekly intervals and killed 1 wk after the last dose. Proliferation and cytokine responses in lymphocytes from Payer's patches and spleen, and antigen specific antibodies in serum were studied. Zn supplementation of ZD dams led to enhanced lymphocyte proliferation and IFN- γ responses in pups ZDZ+. In contrast, Zn supplementation of ZC dams suppressed these responses in pups ZCZ+. Total and DNP-specific IgA responses were lower in pups of the Zn-deficient group compared with the Zn-adequate group. Relative thymus weight was greater in the pups (ZDZ-) of ZD placebo-supplemented dams compared with the other groups at 31 d of age. Prepregnancy and early in utero Zn deficiency affected IgA responses in pups that could not be restored with Zn supplementation during pregnancy. Zn supplementation of ZC dams induced immunosuppressive effects in utero that may also be mediated through milk and persist in the offspring after weaning. J. Nutr. 137: 1037–1042, 2007.

Introduction

Observational and experimental studies have highlighted the importance of zinc (Zn)⁵ status in immune function as illustrated by Zn deficiency being accompanied by an immunodeficiency (1). Severe Zn deficiency has been associated with thymic and spleen atrophy, impaired T cell-mediated responses, and increased susceptibility to infectious diseases (2). Deletion of precursor cells in bone marrow and thymus is believed to result in inability of the host to replenish peripheral lymphocytes, causing increased susceptibility to infectious diseases (2). cDNA array analysis of thymus from moderately Zn-deficient mice demonstrated that aberrations in specific thymic mRNA take place long before T

lymphocyte changes are detectable by Florescent Associated Cell Sorter analysis (3). Impaired immune response to vaccination in experimental animals and in elderly patients with Zn deficiency has been reported (4–7), although the findings are not always definitive (8–10).

Moderate to severe Zn deficiency during pregnancy in experimental animals has been related to adverse effects on offspring, including high rates of fetal resorption, reduced litter size, congenital malformations, reduced splenocyte responsiveness to mitogen (11–13), and reduced serum levels of IgG2a and IgA (14). Supplementation of the neonates at birth with Zn could marginally reduce the immunodeficiency, but the defect persisted to a milder extent in the F2 and F3 progenies (14,15). Observational data in humans during pregnancy show conflicting findings on adverse fetal outcome relating to Zn deficiency, most likely because of the lack of a valid indicator to assess Zn status. Studies of the effect of maternal Zn deficiency on the infant's immune status are scarce. One study of a 6-mo follow-up of infants in Bangladesh demonstrated that only low birth weight (but not normal birth weight) infants of mothers who received Zn supplementation during pregnancy had reduced risk of diarrhea, dysentery, and impetigo compared with the placebo group (16). Another study by the same group documented the benefit to

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⁵ Abbreviations used: AAS, atomic absorption spectrophotometry; BSA, bovine serum albumin; Con A, Concanavalin A; CT, cholera toxin; DNP, dinitrophenol; PP, Peyer's patch; ZC, control dams that received adequate zinc in the diet; ZCZ+, pups of control dams that received zinc supplementation; ZCZ-, pups of control dams that received placebo; ZD, zinc-deficient dams that received diet marginally deficient in zinc; ZDZ+, pups of zinc-deficient dams that received zinc supplementation; ZDZ-, pups of zinc-deficient dams that received placebo; Zn, zinc.

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infant immune status by showing reduction in infectious and diarrheal disease morbidity incidences throughout the 1st period of life (17). However, direct investigations of the effects of Zn deficiency and supplementation during pregnancy on immune function in offspring have not been conducted.

We hypothesized that Zn deficiency-induced immunosuppression in pregnancy is transferred to the offspring that persists after weaning and can be corrected by Zn supplementation during pregnancy. Mild Zn deficiency is more common in human populations. Therefore, we investigated effects of mild Zn deficiency during pregnancy on the development of the immune system in offspring using a rat model. Public health programs of prenatal Zn supplementation do not take into account maternal Zn status, which is an unexplored area. Thus, we further examined Zn-adequate pregnant rats for effects of Zn supplementation on the ability to boost immune response in the offspring after weaning.

Materials and Methods

Rats. The study complied with the Guide for the Use and Care of Laboratory Rats and was conducted under the auspices of Animal Resource Services of the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Virgin female Sprague-Dawley rats ($n = 16$; 7–8 wk old, ~180g) were obtained from Charles River. The rats were maintained in stainless steel hanging cages under constant conditions (22°C, 65% humidity) with a 12-h-dark:light cycle and consumed food ad libitum. After consumption of standard nonpurified rat diet (LabDiet) for a 3-d acclimatization period, rats were randomly assigned to 1 of 2 experimental diets and allowed to consume the food and deionized water ad libitum. We developed the Zn-deficient pregnant rat model, as described previously (18). The Zn-adequate control group (ZC; $n = 8$) was given a diet containing 25 μg Zn/g diet and the other group of rats (ZD; $n = 8$) received a diet marginally deficient in Zn (7 μg Zn/g diet). Rats received these diets for 15 d prior to conception through 21 d of pregnancy and 31 d postparturition. Following pregnancy confirmation, these rats were further divided into 2 subgroups to receive either Zn (1.5 mg Zn in water, Z+) or placebo (water, Z-) 3 times/wk throughout pregnancy (Fig. 1). Zn status in pregnant dams was determined by measuring Zn in serum collected from tail blood (18).

After birth, different groups of pups were designated as follows: ZCZ+, pups of control dams that received Zn supplementation; ZCZ-, pups of control dams that received placebo; ZDZ+, pups of Zn-deficient dams that received Zn supplementation; ZDZ-, pups of Zn-deficient dams that received placebo. At birth, litter size was recorded, but body weight was not measured. Pups of ZD dams were much smaller than those of the ZC group. Postnatal survival was less in the ZD group. Pups were culled to 8 pups/dam at d 3. After weaning, pups received the same diet as their mothers. Pups were killed at d 31 to determine the long-term effects of maternal Zn intake during pregnancy on cellular and mucosal immune function in the offspring. Body weight was first measured at d 10 and thereafter every week. Rats were killed by asphyxiation with CO_2 , body weight was measured, and blood was collected immediately.

Immunization. We immunized pups from 4 treatment groups ($n = 12 \times 4$) via oral gavage at d 10, 17, and 24 with cholera toxin (CT; 10 μg /immunization) and T lymphocyte-dependent antigen, dinitrophenol-bovine serum albumin (DNP-BSA; 50 μg /immunization) in a total volume of 0.1 mL 3% sodium bicarbonate buffer to neutralize gastric acid (Fig. 1). Bicarbonate only was given to pups ($n = 4 \times 4$) as a control.

Tissue and blood collection. We recorded the weight of thymus. Cells were harvested from spleen and single cell suspensions were made by passing through nylon mesh (10- μm pore size) with a rubber spatula; they were then used for cell culture and the proliferation assay. Peyer's patch (PP) lymphocytes were tweezed out from the small intestine, applied to a grinder, and passed through nylon mesh to obtain a single cell suspension

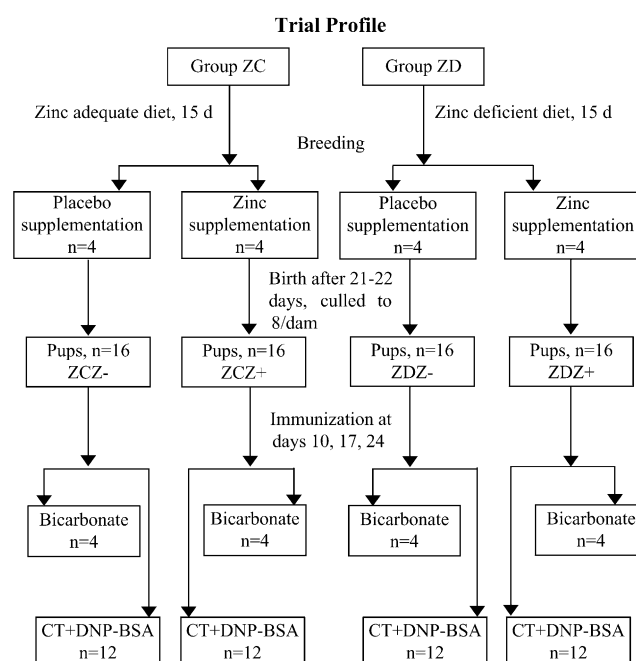


Figure 1 Trial profile demonstrating number of dams in each group that were fed Zn-restricted (7 μg Zn/g diet) or Zn-adequate diet (25 μg Zn/g diet) and were supplemented with either Zn (1.5 mg Zn in water, 3 times/wk) or placebo (water). Pups were immunized via oral gavage at d 10, 17, and 24 with CT (10 μg /immunization) and DNP-BSA (50 μg /immunization) in 0.1 mL of 3% sodium bicarbonate buffer. Only bicarbonate was given to pups as a control.

(19). Samples of intestine and liver were dissected and immediately snap-frozen in liquid nitrogen and frozen at -80°C for Zn analysis. Blood obtained by cardiac puncture was collected into trace element-free vials and serum was separated immediately and frozen at -80°C until analysis.

Mineral analysis. Tissue minerals were measured by atomic absorption spectrophotometry, as described previously (20). Briefly, ~0.2 g of tissues was digested in acid-washed vials with 3.0 mL of 16 mol/L HNO_3 for 48 h. Samples were boiled to a volume of ~1.0 mL and ultra-pure water added to a volume of 5.0 mL. Serum (120 μL) was digested in 1.0 mL of 1 mol/L HNO_3 for 48 h prior to absorption spectrophotometry.

Lymphocyte proliferation response. Mononuclear cells were isolated from spleen and PP in the small intestine and single-cell suspensions were cultured, as previously described (21), using 3 doses of CT and 3 doses of DNP-BSA to measure proliferation to both. Different doses (0.5, 1.0, and 2.0 mg/L) of the mitogen concanavalin A (Con A) was used as control mitogen. Culture supernatants were collected at 72-h postincubation and stored at -70°C until used. Proliferation was assessed by bromodeoxyuridine incorporation using standard methods and commercial reagents (US Biochemicals). Data were expressed as absorbance at 450 nm (reference wavelength, 690 nm).

Cytokines. Cytokines of both the T helper cell type 1, IFN- γ , and T helper cell type 2, IL-4 and IL-10, were measured in the culture supernatant of splenocytes and PP lymphocytes stimulated with Con A, CT, and BSA. We measured cytokines using commercial enzyme immunoassays (R&D Systems). The lowest limit of detection was 10 ng/L for IFN- γ and 1 ng/L for IL-4 and IL-10.

Antigen-specific antibody responses in serum. DNP and CT-specific IgG and IgA, as well as total IgA antibodies, were measured in serum according to the method described previously (22). In brief, polystyrene microtiter plates (Nunc-Maxisorp) were coated with DNP-ovalbumin in carbonate buffer (0.1 mol/L sodium bicarbonate and 5 mmol/L magnesium chloride, pH 9.8) and incubated overnight at 4°C and the standard

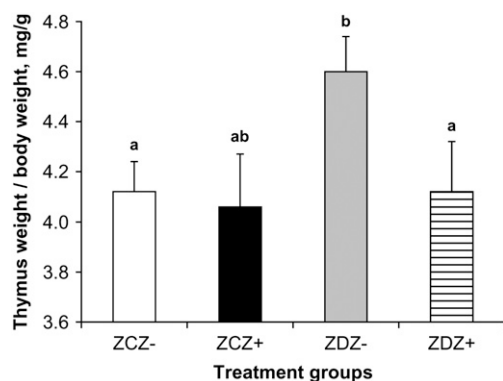


Figure 2 Relative thymus weights of 31-d-old pups of Zn-adequate and Zn-deficient dams supplemented with Zn or placebo. Values represent means \pm SEM, $n = 12$. Means without a common letter differ, $P < 0.05$.

procedure was followed. Data were expressed as mg/L of antibodies. CT-specific responses were measured, as previously described (21).

Statistical analyses. Statistical analyses were conducted using the statistical software packages SIGMASTAT (version 3.1; Jandel Scientific) and SPSS for WINDOWS (release 10; SPSS Institute). When a variable was not normally distributed, an appropriate transformation (e.g. log or square root) was used to better achieve approximate normality. Analyses were performed on the transformed variables to meet the underlying assumptions of the statistical tests used. When the data could not be normalized, nonparametric analysis (rank-sum test) was performed. Differences were significant at $P < 0.05$. Results were expressed as means \pm SE. Data were analyzed using 2-way ANOVA to test for main effects of dietary Zn content and Zn supplementation and their interaction during pregnancy. When the 2-way interaction was significant ($P < 0.05$), 1-way ANOVA was conducted, followed by Tukey's test. Repeated measures ANOVA was used to test body weight data. Body weights on d 10, 24, and 31 were used as within-subject variable with Zn supplementation as the between-subject factor.

Results

Body and thymus weights. Body weight in the ZCZ+ group on d 10 was higher ($P = 0.001$) than in the ZCZ group, but body weights on d 24 and d 31 did not differ (data not shown). Body weight in the ZDZ+ group was higher ($P = 0.001$) on d 10 than in the ZDZ- group. Gain in body weight over the study period was higher in the pups of the ZD groups than in the ZC groups ($P = 0.03$) (data not shown).

Thymus weight was significantly higher in the ZDZ- group than in the ZDZ+ group, but it did not differ between the 2 ZC groups (data not shown). The weight of the thymus in the ZDZ- group was markedly higher than those in the ZCZ- ($P =$

0.001) and ZCZ+ ($P = 0.002$) groups. When corrected for body weight, the thymus weight in the ZDZ- group was higher than in the ZDZ+ and the ZCZ- groups and tended to be higher than the ZCZ+ group ($P = 0.053$) (Fig. 2).

Zn concentrations. At the preconception stage, serum Zn concentrations in ZD and ZC rats were similar, as described earlier, during moderate Zn deficiency in pregnant rats (Table 1) (18). At d 31, pups in the ZC groups had significantly higher concentrations of Zn in serum, liver, and intestinal tissue compared with the ZD groups. However, within the ZC and ZD groups, Zn supplementation did not affect serum or tissue Zn concentrations (Table 1).

Lymphocyte proliferation response. The optimum dose for stimulation with Con A was 1.0 mg/L, although significant differences between the groups were obtained at 0.5 mg/L. Spleen lymphocytes (splenocytes) in the ZCZ+ group had a lower response compared with the ZCZ- group ($P = 0.005$) at 0.5 mg/L Con A and tended to be lower at 2.0 mg/L ($P = 0.06$) (Table 2). However, the ZDZ- and the ZDZ+ groups did not differ at any dose of the mitogen.

PP lymphocytes of the ZDZ+ group tended to have a greater response to 0.5 mg/L Con A than those of the ZDZ- group (data not shown) ($P = 0.053$). However, the proliferation response of PP lymphocytes was lower in the ZCZ+ group (0.34 ± 0.02) compared with the ZCZ- group (0.46 ± 0.03) ($P = 0.015$) at 0.5 mg/L Con A.

Proliferation responses of splenocytes or PP lymphocytes to the immunogens CT or BSA were below the detection limit in most rats (data not shown).

Cytokines in cultured lymphocyte supernatant. Cytokines IL-4 and IL-10 were detectable in only a few rats (data not shown); however, high levels of IFN- γ were obtained in the supernatant. Within the ZD and ZC groups, the levels of IFN- γ in splenocyte cultures were not affected by Zn supplementation. However, IFN- γ levels were higher in the ZDZ+ group than in the ZCZ+ group ($P = 0.01$) (Fig. 3). This may reflect a suppressive effect of additional Zn in the ZCZ+ group in the release of IFN- γ in the culture supernatant. The IFN- γ levels did not differ between the ZDZ- and ZCZ- groups.

In the cultures of PP lymphocytes, low levels of IFN- γ were detected in the supernatant. The IFN- γ levels did not differ between the ZD and ZC groups (data not shown).

Antigen-specific responses. Levels of antibody against CT or BSA were very low and in most cases were below the detection limit (data not shown). However, DNP-specific IgA and IgG1 were measurable in serum.

Total IgA concentrations were higher in pups in the ZCZ- group compared with the ZCZ+ group (Table 3). However, the

TABLE 1 Concentrations of Zn in serum of Zn-adequate and Zn-deficient dams at preconception and in serum and tissues of pups of Zn-adequate and Zn-deficient dams supplemented with Zn or placebo¹

Dams, Preconception	ZC		ZD		2-way ANOVA (<i>P</i> -values)		
Serum, <i>mmol/L</i>	1.66 ± 0.03		1.69 ± 0.06				
	ZCZ–	ZCZ+	ZDZ–	ZDZ+	Dietary Zn	Zn supplement	Interaction
Pups, 31 d old							
Serum, <i>mmol/L</i>	1.65 ± 0.06	1.53 ± 0.09	0.94 ± 0.06	0.79 ± 0.05	0.001	0.82	0.07
Intestine, <i>μmol/g</i>	24.5 ± 0.64	23.6 ± 0.68	20.4 ± 0.57	18.7 ± 0.59	0.001	0.04	0.52
Liver, <i>μmol/g</i>	26.3 ± 0.63	24.9 ± 0.97	23.9 ± 0.64	22.6 ± 1.24	0.009	0.10	0.94

¹ Data are means \pm SE; $n = 12$ (pups) or 8 (dams).

TABLE 2 Lymphocyte proliferation response (bromodeoxyuridine) in splenocytes from pups of Zn-adequate and Zn-deficient dams supplemented with Zn or placebo¹

Con A	ZCZ−	ZCZ+	ZDZ−	ZDZ+	2-way ANOVA (<i>P</i> -values)		
					Dietary Zn	Zn supplement	Interaction
<i>A</i> ₄₅₀							
0.5 mg/L	0.42 ± 0.03 ^a	0.30 ± 0.05 ^b	0.32 ± 0.04 ^b	0.27 ± 0.02 ^b	0.10	0.03	0.05
1 mg/L	0.82 ± 0.09	0.70 ± 0.09	0.63 ± 0.09	0.78 ± 0.09	0.57	0.87	0.14
2 mg/L	0.93 ± 0.10	0.69 ± 0.07	0.70 ± 0.08	0.90 ± 0.11	0.94	0.83	0.01

¹ Data are mean absorbances ± SE, *n* = 12. Means in a row with superscripts without a common letter differ, *P* < 0.05.

total and DNP-specific IgA levels did not differ between the ZDZ+ and ZDZ– groups. Pups of the ZC groups had higher levels of total IgA (*P* = 0.01) as well as DNP-specific IgA (*P* = 0.04) compared with those in the ZD groups. Total IgA levels in the ZCZ– group were significantly higher than the other groups (Table 3). Levels of DNP-specific IgG1 did not differ among the groups.

Discussion

Direct benefits in infant immune status as a result of maternal Zn supplementation have shown reductions in infectious and diarrheal disease morbidity incidences throughout the 1st period of life (17). However, direct investigations of the effects of Zn deficiency and supplementation during pregnancy on immune function in offspring have not been evaluated. Here, we report that Zn deficiency during pregnancy in rats was reflected by changes in immune function of the offspring such that Zn supplementation to Zn-deficient dams restored cellular immune deficits in the offspring but could not restore humoral immunity. Moreover, Zn supplementation to dams with adequate Zn nutriture lead to suppressed cellular immunity in their pups, suggesting that Zn “over-nutrition” may be as important as Zn “under-nutrition” in vulnerable populations.

The immunological consequences of severe Zn deficiency during pregnancy for the fetus have been shown in animal models with reductions in size of spleen and thymus, leading to cellular immune defects (14,23,24) and lower antigen-specific, antibody-mediated responses in offspring (25). Zn supplementation of Zn-

deficient animals and humans restores immune deficiency, including lymphocyte function, delayed hypersensitivity response, and cell-mediated immune responses (26,27). However, studies directly investigating effects of Zn supplementation during pregnancy on immune function in their infants or older children are lacking (17). In the absence of a valid indicator of Zn deficiency, observational studies of Zn supplementation of pregnant women have generated conflicting results. One study showed high IgG levels in cord blood of mothers supplemented with Zn (17). Another study found no effect on immune response to Hib-conjugate vaccine in infants of mothers supplemented with Zn; however, fewer anergic responses to delayed hypersensitivity skin test were reported in low but not normal birth weight infants after maternal Zn supplementation (17,28), suggesting a positive effect of Zn supplementation only in potentially Zn-deficient infants. In this study, we produced mild Zn deficiency in pregnant dams. This condition is relatively common in human populations, especially in developing countries. We found that Zn supplementation of Zn-deficient dams led to increased proliferation response of PP lymphocytes in the d-31 offspring compared with the placebo group. Secretion of IFN-γ in response to a T cell mitogen, con A, was higher in the young pups from Zn-supplemented, Zn-deficient dams compared with the offspring of Zn-supplemented, Zn-adequate control dams. These findings suggest that repletion of Zn status during pregnancy restores the mucosal cellular immune function of offspring and thus has a beneficial effect on the immune system of the progeny.

Zn supplementation has been shown to reverse immune defects seen in Zn deficiency. In contrast, high doses of *in vitro* Zn have been documented to impair T cell function (26,29) and block IFN-α production (30). Only 1 study in elderly humans reported depressed immune function after oral intake of high Zn (31). Reports on the effect of surplus Zn during Zn-adequate pregnancies on human infants are absent and limited in experimental animals. One study in adult female mink reported that excessive Zn supplementation (1000 μg/g) led to achromatrichia, alopecia, lymphopenia, suppressed lymphocyte proliferation response, and reduced growth rate in their offspring (32). The depressed immunity was restored after placing the offspring on an unsupplemented diet for 14 wk. In our study, Zn supplementation of dams fed a Zn-adequate diet resulted in suppressed lymphocyte proliferation response of spleen and PP lymphocytes and reduced IFN-γ production in the offspring, suggesting inhibitory or suppressive effects of excess Zn on T lymphocyte functions. The level of Zn supplementation used in our study was very modest and similar on a body weight basis to amounts that pregnant women would be given. T cells have low intracellular Zn concentrations and are more susceptible to increased Zn levels than other cells (26). High Zn concentration suppressed alloreactivity in mixed lymphocyte culture (33), decreased lymphocyte reactivity to mitogens (34,35), and blocked IFN-α production (30).

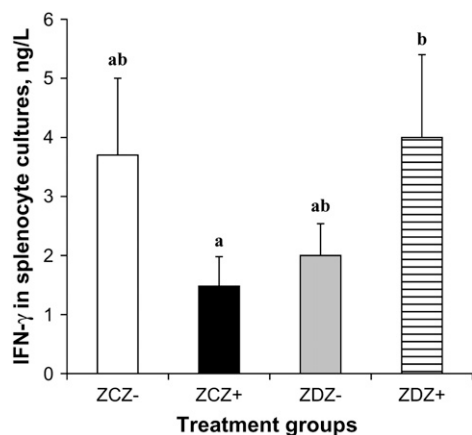


Figure 3 Concentration of IFN-γ in culture supernatants of Con A-stimulated splenocytes from pups of Zn-adequate and Zn-deficient dams supplemented with Zn or placebo. Values represent means ± SEM; *n* = 12. Means without a common letter differ, *P* < 0.05.

TABLE 3 Total and antigen-specific antibodies in serum of pups of Zn-adequate and Zn-deficient dams supplemented with Zn or placebo^{1,2}

Antibody	ZCZ−	ZCZ+	ZDZ−	ZDZ+	Two-way ANOVA (<i>P</i> -values)		
					Dietary Zn	Zn supplement	Interaction
			<i>mg/L</i>				
Total IgA ¹	580 ± 130 ^a	463 ± 220 ^b	27.3 ± 12 ^b	47.1 ± 13 ^b	0.01	0.57	0.02
DNP-IgA	23.3 ± 8	16.6 ± 13	6 ± 2.4	4 ± 1	0.04	0.25	0.80
DNP-IgG1	45 ± 10	166 ± 70	29 ± 4	36 ± 12	0.06	0.06	0.10

¹ Data are means ± SE, *n* = 12. Means in a row with superscripts without a common letter differ, *P* < 0.05.

² Pups were orally immunized with DNP-BSA and CT.

The inhibitory effects of excess Zn may also result from Zn-induced copper deficiency (36,37) and an imbalance in vitamin A homeostasis (38), because both copper and vitamin A are important for T cell function. The findings indicate that excessive Zn supplementation during pregnancy had an adverse carryover effect on the offspring.

Zn deficiency leads to decreased humoral response (1,27) and Zn supplementation restores antibody response. In a study of postpartum marginal Zn deprivation in mouse dams, the ability to mount antibody-mediated responses in suckling pups was reduced (25). Effects of Zn deficiency in dams on the immune responses of pups mediated via milk were reversed by Zn supplementation of the pups. However, in our study, prepregnancy Zn deficiency in rat dams caused a permanent defect imprinted in utero in the humoral arm of immunity that could not be restored even after Zn supplementation to the pregnant dams. Indeed, lowered response to B cell mitogen was reported in the offspring of marginally Zn-deficient monkey dams (39).

One drawback of this study was that oral immunization of suckling rats started at the age of 10 d and given at weekly intervals when the immune system of these pups was still immature (21). The lack of antibody response or lymphocyte proliferation response to specific antigens, CT or BSA, might be due to the immaturity of the mucosal immune system or a result of induced tolerance due to short intervals of immunization.

An interesting observation in this study was the increased size of the thymus in the 31-d-old offspring of ZD dams receiving placebo. Studies in experimental Zn-deficient models have shown that severe Zn deficiency causes thymic atrophy and decreases thymic size (2,27). However, to our knowledge, this is the 1st report to show that mild Zn deficiency during fetal development and continued suboptimal Zn nutriture during infancy resulted in enlarged thymic size in the offspring. Despite having smaller size at birth, a common feature during maternal Zn deficiency in animals (14,24), these young Zn-deficient pups also had higher weight gain by adolescence. The difference in thymus weights remained significant after being corrected for body weight. In humans, thymus size is largest at puberty and is reduced at adulthood to the size at birth. It is possible that the ZDZ– group did not attain the natural size of thymus by adolescence due to delayed maturity. It is also possible that marginally Zn-deficient pups being kept in a relatively sterile environment and nutritional affluence might have had a rapid catch-up growth causing obesity in adult life (40,41).

In conclusion, we found that effects of maternal Zn deficiency and supplementation persisted after weaning, influencing cellular and humoral immune functions of young pups. In addition, Zn supplementation to Zn-deficient dams during pregnancy corrected cellular immune function, whereas that to Zn-adequate dams was immunosuppressive and persisted in the offspring in later life. Humoral immunodeficiency in pups could not be

restored with maternal Zn supplementation. Thus, in the public health context, further research assessing optimum dosage of Zn supplementation during pregnancy, especially linking it to immunological outcome in infants and children, is crucial.

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